

Poster Abstracts, MAS 2013

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***Generating a LacI-Producing Derivative of Borrelia burgdorferi Strain 297-AH130***

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***Introduction***

*An inducible promoter system was developed for Borrelia burgdorferi, but was limited in use because the host strain had lost lp28-1, a plasmid necessary to infect immunocompetent mammalian hosts. We intend to develop a new host strain for this system that has the virulence plasmids to infect immunocompetent hosts stabilized with selectable markers. My goal is to integrate the E. coli lacI gene and an associated antibiotic resistance marker into an intergenic region of lp28-1.*

***Methods***

*We assembled a DNA construct to integrate the E. coli lacI gene and an antibiotic resistance marker into an intergenic region of lp28-1. The construct was used to transform B. burgdorferi via electroporation. The cells were plated and transformants were screened for the insertion and LacI production.*

***Results***

*We performed numerous transformations of B. burgdorferi and we are currently screening colonies for the appropriate insertions as well performing Western blots to screen for LacI production.*

***Conclusion***

*All LacI-producing derivatives will also be screened for lp25, another B. burgdorferi virulence associated plasmid often lost during in vitro cultivation. We will then stabilize lp25 by inserting a different selectable marker and generate a new infective host strain for the B. burgdorferi inducible promoter system.*

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***Identifying Specific Receptor and Ligand Combinations in the PVR Signaling Pathway in Drosophila***

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*Cells communicate to neighboring cells by sending and receiving signals. A ligand secreted by one cell often binds a transmembrane receptor of another cell to send a unique instruction to carry out basic cellular activity. Some related receptors, or isoforms, can be encoded by a single gene. The PDGF/VEGF Receptor (Pvr), a*

receptor in *Drosophila melanogaster*, has three isoforms (PvrA, PvrG, and PvrI) that differ in their extracellular domains, where the ligand binds. Pvr also has three known ligands: Pvf1, Pvf2, and Pvf3. We hypothesize that each of the three ligands will signal through a distinct Pvr receptor isoform. The first phase of the research is to determine which tissues are sensitive to Pvr by overexpressing a constitutively activated receptor using a variety of tissue specific drivers. Preliminary results have shown that the head embryonic tissue is sensitive to Pvr. This tissue will then be used to perform over- and under-expression of different combinations of ligands and receptors to determine which ligand receptor combinations result in signaling.

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### **Miniature Inverted-repeat Transposable Elements in *Chlamydomonas***

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Transposable elements (TEs) have been identified in all known organisms. Although they are usually found in the non-coding regions of DNA, TEs do have the ability to move around in the genome. We are specifically interested in a unique type of TE called a Miniature Inverted-repeat Transposable Element (MITE). To monitor MITE movement, we are developing a screenable assay in *Chlamydomonas*. We intend to block expression of the green fluorescent protein (GFP) with the presence of an interfering MITE. Future movement of the MITE will be easily observable in a simple plate assay due to the fluorescence of the *Chlamydomonas*. Two separate DNA constructs are being created for this assay. In our first construct, a MITE will be located between a promoter and the GFP gene. Anticipating leaky expression due to the small size of the MITE, a second construct will place the MITE inside the GFP gene.

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### **Effects of Psyllium Supplementation on Protein, Triglycerides, Serum Electrolytes and Packed Cell Volume in Grazing Horses**

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Psyllium has been previously used as a supplemental dietary fiber in humans and to treat and prevent sand colic in horses. Research reported that psyllium supplementation decreased triglyceride values in humans and glucose values in humans and horses. Psyllium supplementation has been reported to improve the hydration state of horses. The effect of psyllium on absorption of other nutrients and on feed and water intake has not been studied. The objective of this study was to identify effects of psyllium supplementation on feed and water intake and the absorption of

*nutrients and water in the intestine of horses.*

*Eleven horses, 10 to 18-years old, were used to determine the effects of psyllium supplementation on nutrition and hydration state of the grazing horse. Treatments were: 1) 180 gram psyllium pellet, and 2) an isocaloric control. There were no differences ( $p > 0.5$ ) in chloride and sodium concentrations, PCVs, and water and feed intake. However, horses that received psyllium showed reduced concentrations of triglycerides ( $p = 0.0409$ ) and protein ( $p < 0.001$ ). The lowered protein and triglyceride concentrations in the present study indicate that psyllium supplementation decreases absorption of these nutrients in the horse's intestine.*

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Forest Fire Observation Platform

Jackson Langland

*The original intent of this project was to create a contraption capable of lofting a camera to an altitude where it could relay video to a receiver on the ground. I settled on a rocket assisted glider because it could spend more time at altitude than just a rocket by itself. However as I began to realize exactly how difficult building such a device is extremely complicated. I soon realized that I would first need to create a glider which could fly stably. So far I am still testing designs that will meet this goal. Once I have a sound glider I will begin testing methods to attach a rocket engine to the glider.*

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*Alternative conformations of yeast iso-1-cytochrome c: effects of a gate keeping residue on heme crevice dynamics*

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*Recently, Cytochrome c (Cyt<sub>c</sub>) has been identified in initiating the caspase cascade leading to apoptosis upon release from the mitochondria. Interaction of Cyt<sub>c</sub> with cardiolipin on the mitochondrial membrane requires loss of the native Met80 heme ligation, which in turn enables Cyt<sub>c</sub> to function as a peroxidase and oxidize cardiolipin. Cyt<sub>c</sub> has a decreased affinity to oxidized cardiolipin, and can be released from the membrane and the mitochondria propagating apoptosis. Peroxidase activity requires a dynamic movement of the  $\Omega$ -loop D to open the heme coordination site. In yeast wild-type iso-1-Cyt<sub>c</sub> (yWT) a trimethylated lysine (TmLys) at residue 72 is positioned near the heme crevice in contact with Thr78, Met80 and Ala81. We hypothesize that this steric interaction may act as a gatekeeper for the dynamic*

opening of the  $\Omega$ -loop D enabling Met80 to swing outwards opening up the heme coordination site. We obtained a crystal structure of yeast iso-1-Cytc containing a TmLys72Ala mutation, eliminating steric interactions of residue 72, that demonstrates an open heme coordination site conformation with Met80 swung away from the heme. Using circular dichroism and stopped-flow methods we investigate residue 72 as a gatekeeper of peroxidase activity by monitoring peroxidase activity and cardiolipin/cytochrome c interactions.

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### **Measuring the Impact of Floating Treatment Wetlands in Simulated Waste Water Lagoons**

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Floating Treatment Wetlands (FTWs) are a highly customizable floating substrate that may provide a means of improving water quality. As the name implies, they are designed to float on the water's surface while vegetation grows roots down into the water column. The plants and microbes associated with the FTWs likely remove contaminating nutrients, such as nitrogen, ammonium, and phosphorus from the water body. In conjunction with the City of Billings, Rocky Mountain College is running an experiment to monitor the efficacy of FTWs in simulated wastewater lagoons. The experiment uses twelve, greenhouse lit, 1500 liter, mesocosms, where each aquatic environment is equipped with aeration, circulation, and temperature control. The experiment is being conducted over 30 days, with daily measurements of nine water quality indicators. The twelve mesocosms allow replicates of six unique conditions that test FTW performance in the presence and absence of aeration or circulation. Overall we intend to model the impact of FTWs in typical wastewater lagoons with the intent of conducting a future pilot scale project.

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### **The identification and characterization of a potential target of the F-box protein Cdc4p in *Candida albicans*.**

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*Candida albicans* is a major fungal pathogen of immunocompromised humans. *C. albicans* grows in yeast and filamentous/hyphal forms. Advances have been made in understanding the signaling pathways controlling the switch to filamentous growth, accompanied by changes in protein complement, and their role in virulence. However, the relationship between the signaling pathways, morphogenesis and the cell cycle is

not well understood. The SCF complex ubiquitin ligases and their subunits, specifically the F-box proteins, are traditional cell cycle regulators that add ubiquitin peptides to “target” proteins. The F-box protein Cdc4p does not seem to play a critical role in cell cycle progression in *C albicans*, but is a negative regulator of filamentous growth. To elucidate the mechanism through which Cdc4p regulates morphogenesis, we utilized proteomic approaches to identify potential Cdc4p targets that may be ubiquitinated. We have identified 20 proteins whose levels are altered in *cdc4/cdc4* mutants relative to wildtype cells that are potential Cdc4p targets. These 20 proteins include Cdc19, a pyruvate kinase. To determine if Cdc4p directly interacts with Cdc19, we are utilizing standard two-hybrid and western blots with anti-ubiquitin antibodies. This project is increasing our understanding of the role post-translational modifications, such as ubiquitination, play in fungal pathogenesis.

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#### *How does non-native leaf litter influence soil microbial activity?*

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Non-native species can have serious effects on the cycling of nutrients through ecosystems. Nutrients are typically cycled into the soil as invertebrates and microbes decompose leaf litter. When non-native plants replace natives, the physical and chemical characteristics of the leaf litter entering the system changes, which can change how microbes decompose the litter, which in turn can change how nutrients cycle through the ecosystem. The purpose of this research was to determine if leaf litter from Russian olive (*Elaeagnus angustifolia*) influences soil microbial activity rates. Russian olive is strongly invasive in part because of its nitrogen fixing ability. This ability is of note because it means that Russian olive leaf litter alters the nitrogen content of the soil and may alter microbial activity in the soils it occupies. One hypothesis is that the extra nitrogen content of Russian olive leaf litter will stimulate higher rates of microbial activity and quicker decomposition. An alternative hypothesis is that Russian olive litter may stimulate less microbial activity and decompose more slowly because of its high concentrations of lignin. Preliminary results show a greater microbial activity under Russian olive trees than under native cottonwoods (*Populus spp.*).

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#### *Determining Coliform and E. coli Levels in Pryor Creek*

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*The purpose of this research is to analyze levels of coliform and E. coli bacteria in Pryor Creek. Water samples are taken from Pryor Creek near Pryor, MT on the Crow Indian Reservation in Southeast Montana. There is concern about water quality in*

Pryor Creek. In particular, the local community is concerned about whether there may be contamination of the creek due to its close proximity to a primary treatment waste water lagoon and an old and potentially leaking waste water pipe that crosses over the creek to empty into the lagoon.

Water samples are collected from various sites along Pryor Creek, including near the lagoon, upstream and downstream of the lagoon, and underneath the waste water pipe. Samples are taken from the middle of the creek, or in areas with higher flow rates. Samples are kept on ice until return to the RMC laboratory, where the IDEXX Colilert water testing system is used to quantify coliform and *E. coli* numbers using a Most Probable Number approach. Preliminary results have shown the presence of coliform and *E. coli*, and trends are being examined.

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### ***Fish Passage on Pryor Creek: The influence of barriers on prairie fish assemblages***

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*Fish movements into tributaries are frequently associated with seasonal and life-cycle related events such as foraging, predator evasion, and spawning. While Salmonid fish passage has been well studied, little information exists about the impact of barriers on warm water species. Pryor Creek is a classic large prairie stream that was isolated from the Yellowstone River for over 100 years due to an irrigation barrier. Previous survey work in Pryor Creek identified the stream as a depauperate system with low fish abundance. A flood that destroyed the irrigation barrier in 2011 provided an opportunity to look at species composition and abundance changes before and after barrier removal. Mark and recapture methods and an Index of Biotic Integrity (IBI) were used to examine the biological condition of fish communities in Pryor Creek. In 2012 a total of 19,672 fish were captured, including 18 species. One fish (*Platygobio gracilis*) pit tagged in the Yellowstone River was recaptured in Pryor Creek, confirming fish passage. After the barrier was destroyed IBI scores improved dramatically. The higher IBI scores recorded suggests that the biological integrity has increased with connectivity to the Yellowstone River.*

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### ***Construction of a Disruption Cassette to Knockout the SEC61 Gene in the Diploid Yeast *Candida albicans****

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## **Introduction**

*Candida albicans* is an opportunistic yeast that is the causative agent of many types of infection. In order to become pathogenic, *C. albicans* must secrete proteins, thus a highly functioning secretory pathway is required for pathogenesis. In *Saccharomyces cerevisiae*, the Sec61 protein resides in the endoplasmic reticulum (ER) membrane and is the core component of the pore through which secretory proteins pass from the cytosol into the ER during the first step of the secretory pathway. The *C. albicans* Sec61 protein is 63% identical to that of its *S. cerevisiae* homolog. In order to determine if the *C. albicans* Sec61 protein plays a similar role to that of its *S. cerevisiae* counterpart, a conditional null mutant strain must be constructed.

## **Methods Results and Conclusion**

A disruption cassette was constructed by flanking a selectable marker (ARG4) with sections from each end of the SEC61 gene. The cassette was then verified through sequencing. The disruption cassette allows for homologous recombination into one of the SEC61 alleles and selection for transformants when grown on media lacking arginine. This cassette will be transformed into a *C. albicans* strain with one allele under control of an inducible promoter (MET3), resulting in a conditional null mutant.

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## **Information Transmission and Online Social Networks**

Quincy Riordan

### **Abstract:**

*Online social networks are becoming increasingly important in our daily lives. We use them to connect with each other and pass information much in the same way we do offline: from person to person, or user to user. However, online social networks allow for information sharing on a larger scale, with some users having greater reach than others. What does influence and information transmission in an online social network look like? How far does the information actually go? I chose blog network Tumblr to research these questions. To map the progress of news through Tumblr, I picked two posts of contrasting popularity from President Obama's blog, a key point of his campaign strategy. Using NetDraw, I generated two sociograms of each post, and analyzed the differences between the two. The first post, with moderate popularity, did not travel very far; the post stayed close to the source. The graph of the second post displayed a fuller range of motion through the network; the post traveled far from the source and was shared among users. Further studies of online information transmission could be used to analyze how online influence translates offline in political results and how such influence could be used.*

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## **Identifying Hydrogen Production in *Synechococcus elongates***

*Gereint P. Sis and Mark Osterlund*

*Some bacteria naturally produce minute amounts of hydrogen (H<sub>2</sub>) under certain conditions. Therefore, it may be possible to isolate a rare mutant that generates elevated levels of hydrogen. We plan to intentionally mutagenize *Synechococcus elongates* with the intent of generating that rare mutant. While the mutagenesis is straight forward, the identification of hydrogen production is problematic. Our plan is to create a solid growth media that incorporates trace amounts of platinum nanoparticles. The platinum will act as a catalyst to separate the bacterially produced diatomic hydrogen into individual atoms that attach to the platinum. An oxidizing agent in the growth media will be used to create an acidic environment by stripping the electrons from the hydrogen. In addition, the growth media will contain a color indicator that responds to changes in pH. This process should result in a localized color change in the growth media. Initial challenges of the project include the efficient distribution of platinum nanoparticles, the identification of an oxidizing agent, and selection of an inert color indicator that does not affect bacterial growth.*

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**P-glycoprotein: Purification and Incorporation into Nanodiscs**

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*P-glycoprotein (P-gp), a member of the ATP Binding Cassette (ABC) superfamily, is a drug transporter that effluxes a broad spectrum of therapeutic agents. P-gp is expressed in many tissues important in drug disposition including the intestine, liver, kidneys, and blood-brain-barrier. P-gp acts to decrease drug absorption following oral administration, facilitate elimination from the body, and decrease drug exposure in tissues such as the brain. This 170 kDa protein consists of two similar halves, each composed of 6 helical transmembrane regions and an ATP-binding domain. Genetic variations in the ABCB1 gene that encodes P-gp lead to alterations in P-gp expression and activity, which can affect multidrug resistance and drug disposition. The goals of this study are to biochemically and biophysically characterize P-gp to understand how ABCB1 genetic polymorphisms alter activity. The first step is to optimize purification of P-gp from Sf9 insect cells. Sonication is a technique that uses high frequency sound waves to disrupt cellular membranes from which P-gp can be isolated. We varied sonication times, between 80 and 310 seconds, to determine which time point yielded the largest amount of functional protein. We used a combination of Coomassie Brilliant Blue protein staining to measure P-gp expression and ATP hydrolysis to measure P-gp activity. Our results show that shorter total sonication times resulted in the optimal recovery of functional protein with the ideal time being around 120 seconds. Longer sonication times led to the recovery of twice the amount of protein, but a 75% reduction in ATP hydrolysis activity indicating that purified P-gp was primarily non-functional. The next step is to incorporate purified protein in nanodiscs, which are a discoidal model system that can be easily tailored to resemble the native plasma membrane environment. Nanodiscs are composed of lipids and cholesterol and are held in shape by two belt proteins, which govern the size of the disc. Nanodisc require a distinct ratio of composition materials and the membrane protein of interest to properly assemble. Using published ratios of nanodisc assembly and our current P-gp*



*purification protocol, we are working to optimize the generation of the highest number of P-gp-incorporated nanodiscs. Additionally, we are looking at the effects of various lipids (POPC, DMPC and E. coli total lipid extract) on the nanodisc membranes and the efficiency of P-gp incorporation. We are also looking at the effects that two different nanodisc purification techniques (SM-2 Bio-Beads and linear dialysis) have on aggregate formation during assembly. After optimizing P-gp purification and incorporation into nanodiscs we plan to conduct single-molecule fluorescence spectroscopy studies to determine how ABCB1 genetic polymorphisms can lead to differences in activity and dynamics. Understanding these differences as related to their polymorphisms can potentially lead to a method to reverse multidrug resistance and increase drug disposition.*

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*Phenotypic and Genetic Characterization of a Hybrid Phoxinus Community in a Montana Prairie Stream*

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*Two sexually reproducing cyprinid fish, the redbelly (*Phoxinus eos*) and finescale dace (*P. neogaeus*), hybridized to form an all-female, asexual species (*P. eos-neogaeus*). These asexual females reproduce through gynogenesis, generating diploid eggs that are activated by redbelly or finescale sperm. Such a mechanism should alleviate the reproductive cost of males and should result in the hybrids outnumbering the coincident redbelly population. However, in Montana, redbelly and hybrids coexist in similar ratios. Sometimes, hybrid eggs fertilized by redbelly sperm can produce sexually reproducing offspring that resemble redbelly dace morphologically, but not genetically; because the original mother in the redbelly-finescale hybridization event was finescale, all hybrids, as well as all of their offspring, have finescale mitochondrial DNA. Sexually reproducing redbelly dace from hybrid mothers are called “cybrids” and can be distinguished from true-breeding redbelly dace by their finescale mitochondrial DNA. Dace were collected from Currant Creek in the Musselshell drainage in south-central Montana and were characterized both phenotypically and by analyzing nuclear and mitochondrial DNA by Polymerase Chain Reaction (PCR). Finding dace with nuclear redbelly DNA and finescale mitochondrial DNA would allow the construction of a model explaining how hybrids repopulate the “redbelly” population and exist in similar ratios.*